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(54) Title of the invention: SOLUTION OF KERATIN IN ORGANIC SOLVENT AND PROCESS FOR ITS PREPARATION

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SPECIFICATION

1. Title Of The Invention

Solution of keratin in organic solvent and process for its preparation

2. Claims

(1) A process for preparing a solution of keratin in an organic solvent, wherein a keratin-containing substance is reduced in at least one organic solvent selected from halogen-substituted C_2~C_7 aliphatic alcohols and alkoxy alcohols with a water content of not more than 50 wt%, represented by the general formula:

$$RO-(CH_2)_nOH \qquad [I],$$

(wherein R is a C_1~C_3 alkyl group and the subscript *n* is an integer of 2 to 4).

(2) A solution of keratin in an organic solvent prepared by dissolving 1.0~3.0 wt% of keratin in at least one organic solvent selected from halogen-substituted C_2~C_7 aliphatic alcohols and alkoxy alcohols with a water content of not more than 50 wt%, represented by the general formula:

$$RO-(CH_2)_nOH \qquad [I],$$

(wherein R is a C_1~C_3 alkyl group and the subscript *n* is an integer of 2 to 4).

3. Detailed Explanation Of The Invention

[Field Of Industrial Application]

The present invention relates to a solution of keratin in an organic solvent and to a process for its preparation that does not involve irreversible disulfide bond denaturation. The present solution of keratin in an organic solvent is used in the manufacture of various industrial products, such as keratinous polymer membranes, films, fibers, sponges, etc.

[Prior Art]

Keratin, which is present in hair, fur, feathers, and other animal tissues as a structural protein, has long been regarded as a promising raw material for industrial materials such as membranes, fibers, etc.

However, keratin is either insoluble or poorly soluble in ordinary solvents. For this reason, using keratin from natural raw materials requires a thorough conversion to short-molecules[ii] by decomposition, reduction treatment of disulfide bonds in keratin, or irreversible protection of the produced thiol groups by chemical treatment, etc. Namely, keratin is used in the form of hydrolysates produced by treating keratin-containing natural materials with strong acids or alkalis; aqueous solutions of keratin obtained by using a combination of reducing agents and protein denaturants, such as high-concentration aqueous solutions of urea, to carry out the reductive cleavage of disulfide bonds in keratin, yielding thiol groups; keratin derivatives obtained by chemical modification with monoiodoacetic acid and sodium sulfite/sodium tetrathionate; or aqueous solutions of keratin obtained by conversion to short molecules based on proteolytic enzymes and reductive cleavage.

[Problems The Invention Seeks To Eliminate]

However, keratin produced by renaturation from proteins obtained by oxidation treatment requires complicated chemical treatment while the rate of recovery is conspicuously low. In addition, during the reduction treatment, a large amount of various denaturants have to be used as solubilizing adjuvants, which renders the procedure extremely complex. For instance, after dissolving keratin in a solvent such as water or methanol, ethanol, amide, etc. in which water is used as the main ingredient, under neutral or basic conditions in the presence of reducing agents such as thiols and protein denaturants such as urea, it is necessary to add surface active agents or carry out the addition of iodoacetic acid to sulfhydryl groups unstable to oxidation and conduct a carboxymethylation reaction. In addition, insoluble substances must be removed in the process of the reaction, and urea and other denaturants have to be eliminated via dialysis. Such dialysis requires a large amount of water and the procedure takes a long time.

Therefore, the above-described prior-art methods of extraction of keratin from keratin-containing substances (1) required a large amount urea and other protein denaturants, solubilizing adjuvants producing surface active effects, and reducing agents, and comprised the operation of dialysis, which was not economically efficient. The procedures used in the methods were complicated and required treatment over an extended period of time, increasing the cost of the end product. In addition, (2) pollution prevention measures had to be taken to treat the large amount of waste water produced in the process of extraction. Furthermore, (3) the use of the produced keratin was limited because it was difficult to mix the aqueous solutions of keratin with organic solvent-soluble substances.

[Means For Eliminating The Problems]

Therefore, the present inventor conducted in-depth investigations into methods of extracting keratin from natural keratin-containing materials without using protein denaturants. As a result, to his surprise, he discovered that keratin can be easily extracted without using any protein denaturants by treating keratin-containing substances in special organic solvents in the presence of reducing agents, and thus arrived at the present invention.

Namely, the present invention provides (1) a process for preparing a solution of keratin in an organic solvent, wherein a keratin-containing substance is reduced in at least one organic solvent selected from halogen-substituted C_2~C_7 aliphatic alcohols and alkoxy alcohols with a water content of not more than 50 wt%, represented by the general formula:

$$RO-(CH_2)_nOH \qquad [I],$$

(wherein R is a C_1~C_3 alkyl group and the subscript *n* is an integer of 2 to 4) and (2) a solution of keratin in an organic solvent prepared by dissolving 1.0~3.0 wt% of keratin in at least one organic solvent selected from halogen-substituted C_2~C_7 aliphatic alcohols and alkoxy alcohols with a water content of not more than 50 wt%, represented by the general formula [I].

The keratin-containing substances used in the present invention may be any substances containing true keratin. In addition to keratin powder, preferred sources include, for instance, hair, wool[iii], horses, cows, feathers from chicken and other birds, horns and hooves from cows, etc.

Additionally, keratin powders may be prepared in accordance with various publicly known methods (for instance, see T.T. Sun and H. Green, J. Biol. Chem., 253, 2053-2060 (1978)).

Among the organic solvents used in the present invention, 2-methoxy alcohol is suggested as a solvent that is representative of the alkoxy alcohols of the general formula [I]. In addition, chlorine- and fluorine-substituted alcohols, which are preferably C_2~C_5 alcohols, are used as the above-mentioned halogen-substituted C_2~C_7 aliphatic alcohols, with 2-chlorethanol, 2,2,3,3-tetrafluoropropanol, and 2,2,2-trifluoroethanol suggested as the representative alcohols. The organic solvents can be used as is, or by adding water to them so as not to exceed 50 wt%. In addition, so long as the effects are not impaired, other organic solvents can be added to the organic solvents. The amount of the organic solvents used should preferably be 10 to 30 times the amount of the keratin-containing substance by weight ratio.

The present invention uses reducing agents capable of reducing disulfide bonds in the keratin of typical keratin-containing substances into thiol groups. 2-mercaptoethanol, thioglycolic acid, toluene-ω-thiol, dithiothreitol, dithioerythritol, and other thiols; tripropylphosphine, tributylphosphine, and other trialkylphosphines; sodium hydrogensulfite, and other inorganic reducing compounds, etc. are suggested as such reducing agents. These reducing agents reduce the disulfide bonds of the keratin contained in the keratin-containing substances and promote conversion to thiol groups.

The amount of the reducing agents is 0.05 to 0.50 mol per 10g of the keratin-containing substance, and, more preferably from the standpoint of reaction efficiency and economic efficiency, 0.05 to 0.20 mol per 10g of the keratin-containing substance.

In order to obtain keratin from the above-mentioned components, the keratin-containing substance is immersed in an organic solvent containing a reducing agent and subjected to vibration for 1 to 24 hours under heating at 30~100°C. The reaction is brought to completion within 28~48 hours at room temperature, within about 24 hours at 50°C, and within 1~2 hours at 100°C. The thus obtained reaction product is subjected to filtration and centrifugal separation to remove insoluble matter, yielding a solution of keratin in an organic solvent.

The mechanism of solubilization of such keratin is believed to be based on the reduction of the disulfide bonds in the keratin and conversion to thiol groups. Because the action of oxygen and other oxidizers in the air on the solution of keratin in an organic solvent restores disulfide bonds and the viscosity of the solution gradually increases, the solution should be stored at a low temperature, under an atmosphere of an inert gas such as nitrogen.

In addition, the reduction and extraction of keratin, in particular, in case of extracting keratin from tissues such as tough nails and wool, is preferably enhanced by finely dividing the raw materials and adding a surface active agent as a solubilization adjuvant in the amount of 10~15 wt% based on the amount of the keratin-containing substance.

Suggested compounds include alkylsulfuric acid salts (for instance, sodium dodecylsulfate), alkylsulfuric ester salts, phosphoric acid ester salts of fatty acid alcohols, sulfosuccinic acid ester salts, and other anionic surface active agents;

cationic surface active agents represented by the following formula:

[*See structure on page 297 – trans.*]

(where one or two groups among R^1, R^2, R^3, and R^4 are linear or branched C_8~C_{20} alkyl groups or hydroxyalkyl groups, and the remaining groups are C_1~C_3 alkyl groups or hydroxyalkyl groups, or benzyl groups, X is a halogen atom or a C_1~C_2 alkylsulfuric acid group or an aromatic quaternary amine salt such as alkylpyridium halide);

N-carboxymethyl derivatives of fatty acid amines, N-sulfoalkylated derivatives, imidazoline sulfonic acid, and other betaine-type amphoteric surface active agents (where the hydrophobic groups are primarily C_{12}~C_{14} alkyl groups or acyl groups, and the counter ions are alkali metals etc.);

and polyoxyethylene alkyl ether based, fatty acid ester based, polyethylene imine based, polyglycerin ether based, ester based and other nonionic surface active agents (where the hydrophobic groups are primarily C_{12}~C_{14} alkyl groups or acyl groups).

The thus obtained keratin has 5 to 10 cysteine groups and 0.5 to 2 cystine groups per 100 residual amino acid groups, its molecular weight being 10,000 to 13,000.

The resulting organic solvent solution is used to form various polymer membranes, films, fibers, sponges, etc. using publicly known film-forming and molding methods.

[Application Examples]

Next, the present invention is explained more specifically by referring to application examples.

Application Example 1

A mixture of 10g chicken feathers and 200 ml 2-methoxyethanol was de-aerated and subjected to nitrogen substitution. Next, 10g of 2-mercaptoethanol were added in a stream of nitrogen gas and the container was hermetically closed. After stirring the mixture for 24 hours at 55°C, the reaction solution was cooled to room temperature. Impurities were removed from the reaction solution by filtration, yielding 180 ml of the target solution of keratin in an organic solvent, i.e. 2-methoxyethanol, which contained a small amount of 2-mercaptoethanol.

To remove the solvent, 10g of said solution were placed in cellophane dialysis tubing and dialysis was carried out using 2-mercaptoethanol (0.3%) as the external dialysate. Next, 0.15~0.23g of keratin powder were obtained by freeze-drying the contents of the tubing. Accordingly, the keratin concentration of said organic solvent solution was assumed to be 1.5~2.3%. When the above-mentioned keratin powder was subjected to amino acid analysis, it was found to contain 5.5 cysteine groups and 1.3 cystine groups per 100 residual amino acid groups. In addition, polyacrylamide electrophoresis showed that its main component was a protein with a molecular weight of 15,000 to 70,000.

Application Example 2

A mixture of 10g chicken feathers, 200 ml 2-chlorethanol, and 15 ml water was de-aerated and subjected to nitrogen substitution. Then, 10g of 2-mercaptoethanol were added in a stream of nitrogen gas and the container was hermetically closed. After stirring the mixture for 24 hours at 55°C, the reaction solution was cooled to room temperature. Impurities were removed from the reaction solution by filtration, yielding the target solution of keratin in an organic solvent, which contained 2-chlorethanol and a small amount of 2-mercaptoethanol.

The solvent was removed in the same manner as in Application Example 1 and 0.23~0.27g of keratin powder were obtained by freeze-drying. Accordingly, the keratin concentration of said

organic solvent solution was assumed to be 2.3~2.7%. Additionally, polyacrylamide electrophoresis showed that its main component was a protein with a molecular weight of 15,000 to 70,000.

Application Example 3

A mixture of 10g wool, 3g sodium dodecylsulfate, and 200 ml 2-chlorethanol was de-aerated and subjected to nitrogen substitution. Next, 10g of 2-mercaptoethanol were added in a stream of nitrogen gas and the container was hermetically closed. After stirring the mixture for 2 hours at 100°C, the reaction solution was cooled to room temperature. Impurities were removed from the reaction solution by filtration, yielding the target solution of keratin in an organic solvent, which contained 2-chlorethanol and a small amount of 2-mercaptoethanol.

The solvent was removed in the same manner as in Application Example 1 and 0.18~0.20g of keratin powder were obtained by freeze-drying. Accordingly, the keratin concentration of said organic solvent solution was assumed to be 1.8~2.0%. When the keratin powder was subjected to amino acid analysis, it was found to contain 8 cysteine groups and 2 cystine groups per 100 residual amino acid groups. Additionally, polyacrylamide electrophoresis showed that its main component was a protein with a molecular weight of 10,000 to 70,000.

Application Example 4

5g of the keratin powder obtained from wool in accordance with the known method was soaked in 100 ml of a mixture of 2-methoxyethanol and 2-chlorethanol (volume ratio: 1:1), and the mixture was repeatedly de-aerated and subjected to nitrogen substitution, whereupon 5g of 2-mercaptoethanol were added thereto. The mixture was subjected to agitation for 10 hours at 45°C. Impurities were removed from the resultant reaction solution by centrifugal separation, yielding the target solution of keratin in an organic solvent as the supernatant.

The solution was subjected to dialysis in the same manner as above and 0.19~0.21g of keratin powder were obtained by freeze-drying. Accordingly, the keratin concentration of said organic solvent solution was assumed to be 1.9~2.1%. When the keratin powder was subjected to amino acid analysis, it was found to contain 8 cysteine groups and 2 cystine groups per 100 residual amino acid groups. Additionally, polyacrylamide electrophoresis showed that its main component was a protein with a molecular weight of 10,000 to 70,000.

Application Example 5

A container containing 1g chicken feathers, 20 ml 2,2,3,3-tetrafluoropropanol, and 1.5g 2-mercaptoethanol was hermetically closed and subjected to vibration for 20 minutes at 50°C. After cooling the reaction solution to room temperature, impurities were removed from it by filtration, yielding the target solution (about 20 ml) of keratin in an organic solvent, i.e. a fluorine-containing alcohol, which contained a small amount of 2-mercaptoethanol.

5 ml of said solution were placed in cellophane dialysis tubing and dialysis was carried out using 0.3% 2-mercaptoethanol as the external dialysate. Next, 0.13g of keratin powder were obtained by freeze-drying the contents of the tubing. Accordingly, the keratin concentration of said organic solvent solution was assumed to be 2.6%[iv]. When the above-mentioned keratin powder was subjected to amino acid analysis, it was found to contain 2 cysteine groups and 6 cystine groups per 100 residual amino acid groups. In addition, polyacrylamide electrophoresis showed that its main component was a protein with a molecular weight of 15,000 to 70,000.

Comparative Example 1

Table 1 lists the results of tests, in which keratin extraction was carried out in the same manner as in Application Example 1 with the exception of using the various solvents listed below instead of 2-methoxyethanol.

Table 1

Solvents	Keratin Raw Material	Temperature (°C)	Hours (hr)	Keratin Extraction Yield
Methanol	Chicken feathers	50	24	0%
Ethanol	"	50	24	Trace amount (not more than 3%)
"	Hair	60	24	0%
"	Wool	60	24	"
Chloroform	Chicken feathers	50	24	"
Dioxane	"	50	24	"
Tetrahydrofuran	"	50	24	"
Benzene	"	50	24	"

[Effects Of The Invention]

The preparation process of the present invention does not require protein denaturants, as did prior-art methods. For this reason, there is no need for dialysis after the reduction treatment. In addition, the present invention allows for obtaining organic solvent solutions containing keratin in a high concentration of 1~3%. The present solution of keratin in an organic solvent has a wide range of uses as a result of containing keratin in a high concentration.

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Translator's Notes

[i] *Other readings of this personal name include Hiro, Hiromu, Hirotsugu, Tooru. – trans.*

[ii] *While "conversion to short molecules" is a literal translation of the term in the original, its pronunciation is the same as that of "conversion to single molecules." – trans.*

[iii] *Literally, "sheep fur." – trans.*

[iv] *Sic, obviously a typographical error. – trans.*